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Improved assessment of bone turnover by the PTH-(1-84)/large C-PTH fragments ratio in ESRD patients

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Improved assessment of bone turnover by the PTH-(1-84)/large C-PTH fragments ratio in ESRD patients.

Background. The "intact" parathyroid hormone (PTH) assay recognizes PTH-(1-84) as well as amino terminally truncated PTH fragments, that is, large carboxyterminal PTH fragments (C-PTH fragments). The present study investigated whether the use of the plasma PTH-(1-84)/C-PTH fragment ratio enhances the noninvasive assessment of bone turnover in patients on dialysis.

Methods. Bone biopsies and blood samples for determinations of routine indices of bone turnover and PTH peptides were obtained in 51 adult patients on dialysis not treated with drugs affecting bone such as vitamin D or corticosteroids. Blood levels of large C-PTH fragments were calculated by subtracting PTH-(1-84) from "intact" PTH. Patients were classified according to their levels of bone turnover based on histomorphometrically obtained results of activation frequency. Prediction of bone turnover by the various blood indices was done by using proper statistical methods. In addition, hypercalcemia was induced by calcium gluconate infusion in a subset of patients, and levels of PTH-(1-84), "intact" PTH, and PTH-(1-84)/C-PTH fragment ratio were determined.

Results. The PTH-(1-84)/C-PTH fragment ratio was the best predictor of bone turnover. A ratio >1 predicted high or normal bone turnover (sensitivity 100%), whereas a ratio <1 indicated a high probability (sensitivity 87.5%) of low bone turnover. Calcium infusion resulted in decrease in PTH-(1-84)/C-PTH fragment ratio.

Conclusions. The PTH-(1-84)/C-PTH fragment ratio predicts bone turnover with acceptable precision for biological measurements. Moreover, a change in serum calcium levels is one of the regulators of the relative amount of circulating PTH-(1-84) and its large C-PTH fragments.

Virtually all patients with end-stage renal disease (ESRD) develop mineral and bone abnormalities secondary to the loss of kidney function. These abnormalities have a substantial impact on the morbidity and mortality of these patients. Despite an initially common pathogenetic pathway, there are no uniform histopathologic changes among the patients. Patients on dialysis present either with various degrees of secondary hyperparathyroidism, with or without mineralization defect, resulting in high or normal bone turnover or with adynamic bone characterized by low bone turnover. Distinction between high or normal versus low bone turnover is essential because these entities require divergent therapeutic approaches, that is, indication for vitamin D and level of dose aggressiveness, use of calcium- versus non–calcium-containing phosphate binders, and choice of calcium dialysate concentration. Currently, histologic analysis of bone after tetracycline labeling remains the gold standard for assessment of bone turnover. However, this technique is not always available. Physicians generally rely on noninvasive methods, particularly on blood concentrations of intact parathyroid hormone (PTH). However, the level of blood PTH that predict low or high-normal bone turnover has not been clearly established.

In the early 1990s, it was advocated that adequate control of secondary hyperparathyroidism, that is, normal bone turnover, is achieved when blood levels of intact PTH are between one and four times the upper limit of normal range [1–5]. However, we and others found low bone turnover on bone histology in patients on dialysis with intact PTH levels of approximately 10 times the upper limit of normal [6, 7]. Because various degrees of superimposed bone aluminum accumulation may have contributed to these discrepancies between studies, we recently revisited the value of intact PTH levels for assessment of bone turnover in 157 patients on dialysis (abstract; Monier-Faugere et al, *J Am Soc Nephrol* 11:554A, 2000) [8]. These patients were given exclusively calcium salts for phosphate binding and did not show any histologically proven aluminum bone accumulation. We found that 89.2% of patients with intact

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PTH levels ≤100 pg/mL had low bone turnover, whereas 89.4% patients with levels ≥500 pg/mL had high or normal bone turnover. However, in the majority of the patients, that is, in patients with intact PTH between 100 and 500 pg/mL, low bone turnover was observed in 59% and high or normal in the remaining 41% of the patients.

The characteristics of the commercially available kits for determinations of intact PTH may account, at least in part, for the relative poor predictability of intact PTH in assessing bone turnover. Indeed, it has been demonstrated that two immunoreactive components can be detected with available assays for intact PTH [9]. One component comigrates with synthetic human PTH-(1-84), whereas the other non-(1-84) component is more hydrophilic and accumulates in renal failure, accounting for 40 to 60% of the total immunoreactivity in ESRD patients [9]. Subsequently, it was shown that the non-(1-84) PTH molecule comigrates closely with the synthetic human 7-84 PTH fragment [10]. Recently, a novel immunoradiometric assay was developed that exclusively detects the full-length 1-84 but not PTH fragments [11, 12]. Combining the results employing this new PTH-(1-84) assay with high-performance liquid chromatography (HPLC) profiles, it was demonstrated that the non-(1-84) PTH consists of amino terminally truncated PTH fragments, that is, large carboxyterminal PTH fragments (C-PTH fragments) [12], likely PTH-(7-84).

The C-PTH fragments have long been considered inactive peptides. However, it has been shown that they regulate alkaline phosphatase, osteocalcin, collagen α1(I), and insulin growth factor binding protein-5 in rat and human osteoblast-like cells [13–16]. They also stimulate the proliferation and activity of osteoclasts [17] and stimulate activity of hypertrophic chondrocytes [18, 19]. Also, differences in the action of PTH-(1-84) and PTH-(1-34) on various cells have been noted [20–23], pointing to a role of the C-PTH fragments in the activity of PTH. In thyroparathyroidectomized rats, the administration of human PTH-(7-84) alone or in a mixture of other C-PTH fragments such as PTH-(39-84) and PTH-(53-84) antagonized the calcemic response elicited by PTH-(1-84) [24, 25]. Moreover, several binding studies have demonstrated the presence of C-PTH receptors in kidney and bone cells [13, 25–31], especially in osteocytes [31].

Because the large C-PTH fragments contain portions of PTH essential for binding to either the PTH-1 receptor [24, 32–36] or the C-PTH receptor [13, 25–31], the large C-PTH fragments have the potential to antagonize PTH-(1-84) action on bone.

In the present study, we assume that endogenous C-PTH fragments in blood of patients on dialysis are antagonists of the effects of PTH-(1-84) on bone turnover. We hypothesize that the C-PTH fragments act as a negative biologic regulator of the stimulatory effects of PTH-(1-84) on bone turnover, and thus, the PTH-(1-84)/C-PTH fragment ratio has more predictive power in distinguishing low and high bone turnover than the use of PTH-(1-84) alone.

The present study was undertaken (*1*) to test the previously mentioned hypothesis and (*2*) to determine the effects of hypercalcemia on the PTH-(1-84)/C-PTH.

METHODS

Patients

Patients were recruited prospectively from local dialysis clinics during the years 1999 to 2000. The inclusion criteria were age above 18 years and willingness to undergo a bone biopsy and blood drawing. The exclusion criteria were a history of past or present treatment with aluminum phosphate binders, treatment with calcitriol or medications known to affect bone metabolism (diphenylhydantoin, glucocorticoids, cyclosporine) during the last six months, systemic illnesses or organ diseases other than diabetes that may affect bone metabolism (that is, gastrointestinal diseases, liver disease, malignancies, tuberculosis, acquired immunodeficiency syndrome, chronic alcoholism, drug addiction, failed transplant or parathyroidectomy within the last six months, participation in other studies), or tetracycline allergy.

One hundred thirty-five patients were screened. Sixty-five were eligible and 51 agreed to participate in the study; informed consents were signed. There were 29 men and 22 women with a mean age of 47 ± 3 and 43 ± 3 years, respectively. Thirty-two patients were on hemodialysis (HD), and 19 patients were on chronic ambulatory peritoneal dialysis (CAPD). The mean duration on dialysis treatment was 25.6 ± 3.0 months (2 months to 7 years). Underlying kidney diseases were hypertensive nephropathy ($N = 18$), diabetes mellitus ($N = 12$), glomerulonephritis ($N = 8$), interstitial nephritis ($N = 7$), miscellaneous nephropathies ($N = 5$), and unknown origin ($N = 1$). HD patients were dialyzed three times a week for three or four hours. Patients on CAPD underwent four to five 2 to 2.5 L exchanges per day. Calcium dialysate was 2.5 mEq/L. Only routine dialysis support medications were given, including calcium salts for phosphate binding.

Protocol

After signing the consent form, patients were scheduled to undergo an iliac crest bone biopsy. Before bone biopsy, patients received double tetracycline labeling of bone as previously described [37]. During the week before bone biopsies, on days when bone labels were not administered, blood samples were obtained after a 12-hour fast for measurement of circulating calcium, phosphorus, intact PTH, PTH-(1-84), bone-specific alkaline phosphatase (BSAP), and osteocalcin levels. In a subset of six patients, two to four weeks after the bone biopsy,

in vivo dynamic tests of parathyroid gland function, that is, effects of hypercalcemia on PTH peptides, were performed.

Bone biopsy, mineralized bone histology, and bone histomorphometry

Anterior iliac crest bone biopsies were done under local anesthesia and conscious sedation. Bone samples were obtained with the one-step electrical drill technique (Straumann Medical, Waldenburg, Switzerland) as previously described [37].

Bone samples were processed undecalcified as previously described [38]. Sections were stained with the modified Masson-Goldner trichrome stain [39], the aurin tricarboxylic acid stain [40], and solochrome azurin [41]. Unstained sections were prepared for phase contrast and fluorescent light microscopy.

Histomorphometric analysis of bone was done at standardized sites in cancellous bone using the semiautomatic method (Osteoplan II; Kontron, Munich, Germany) at a magnification of ×200 [42, 43]. Activation frequency a parameter, which includes both bone formation and resorption, was measured for determination of bone turnover. In the present study, there was a strong relationship between activation frequency and bone formation rate/bone surface (BFR/BS; $r = 0.96$, $P < 0.001$).

Induction of hypercalcemia

Serum calcium was raised during a two-hour infusion of 10% calcium gluconate (9.3 mg Ca^{2+}/mL) according to the method of Ramirez et al [44]. The initial dose of calcium was 2 mg/kg · hour and was increased thereafter by 1 mg/kg · hour every 20 minutes. Blood was collected 30, 15, and 0 minutes before infusion and every 10 minutes thereafter for determinations of serum ionized calcium, intact PTH levels, and plasma PTH-(1-84) concentrations.

Biochemistry

Serum calcium and phosphorus were measured by standard laboratory techniques. Ionized calcium was determined using a Radiometer Copenhagen (Westlake, OH, USA). Plasma intact PTH levels were determined with the IRMA assay for intact PTH (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The reference range is 15 to 65 pg/mL. The intra-assay and interassay coefficients of variation were 3.4 and 5.6%, respectively. Plasma PTH-(1-84) was determined with the IRMA assay using a radiolabeled detection antibody specific for the first amino acid from the N-terminal site (Whole PTH™; Scantibodies, Inc., Santee, CA, USA) [11, 12]. The reference range of the assay is 7 to 36 pg/mL based on over 120 normal controls [12]. Intra-assay and interassay coefficients of variation were <5 and <7%, respectively. Plasma concentrations of the C-PTH fragments were determined by subtracting the PTH-(1-84) circulating levels from the results of the intact PTH. The PTH-(1-84)/C-PTH fragments ratio was then calculated. Serum osteocalcin levels were measured with the IRMA Human Osteocalcin 100T kit (Nichols Institute Diagnostics). The reference range was 2.0 to 10.0 ng/mL. The intra-assay and interassay variations were 5.3 and 5.7%, respectively. Serum BSAP levels were determined using an IRMA assay (Tandem-R Ostase; Hybritech, San Diego, CA, USA). Reference ranges are 3.9 to 20.9 μg/L for men and 2.9 to 20.1 μg/L for women. The intra-assay and interassay coefficients of variation were 6.7 and 8.1%, respectively.

Statistical analysis

Patients were classified as having high or normal bone turnover if activation frequency was above the lower limit of reference range obtained from bone samples of normal age- and gender-matched volunteers processed and analyzed in our laboratory (>0.42 $year^{-1}$). In the same manner, patients were classified as having low bone turnover if activation frequency was below 0.42 $year^{-1}$.

Results are expressed as mean ± SEM. All statistical tests were two sided. An assigned significance level of 0.05 was used. Normality of distribution was assessed by the Lilliefors test, and homogeneity of variance was tested with the Levene test. Adequate transformations of the data were done for serum calcium (reciprocal) and plasma intact PTH (square root) [45]. Comparisons of continuous values between bone turnover groups were performed by the Student *t* test. The chi-square test was used for categorical variables. Comparisons between results of CAPD and HD patients were done using the Student *t* test. Pearson's coefficients of correlation were obtained between activation frequency and demographic and biochemical variables. Logistic regression was performed to detect predictive factors of activation frequency. Computations and analyses were performed using SPSS 7.5 software package for Windows (SPSS Inc., Chicago, IL, USA).

Post-test probability parameters (sensitivity, specificity, predictive value positive and negative), receiver-operator characteristics (ROC) curves, and areas under the curves were obtained using Analyse-It software for Microsoft Excel, version 1.5 (Analyse-It Software, LTD, Leeds, UK). ROC curves are a plot of the true positive rate (sensitivity) against false positive rate (1 − specificity), and the area under the curve is a measure of test accuracy. Comparisons between the areas under the curves were done with the same software using the Hanley and McNeil methods [46]. In addition, Youden indices (sensitivity + specificity − 1) were calculated for determination of the best cut-off level of a biochemical parameter, which provides the best threshold for diagnosis of a disease, in the present study low bone turnover [47].

Table 1. Demographics, clinical, and biochemical characteristics of 51 chronically dialyzed patients according to levels of bone turnover

	Low bone turnover	High or normal bone turnover
Number of patients	28	23
Age *years*	50 ± 3	39 ± 3[a]
Patients on HD/CAPD	16/12	16/7
Male/female	18/10	11/12
Diabetic patients	8/20	4/18
Duration on dialysis *months*	26 ± 4	25 ± 4
Serum calcium *mg/dL*	9.3 ± 0.2	9.0 ± 0.2
Ionized calcium *mEq/L*	4.85 ± 0.11	4.70 ± 0.08
Serum phosphorus *mg/dL*	6.1 ± 0.4	7.1 ± 0.5
Serum bone-specific alkaline phosphatase *μg/L*	19.8 ± 3.45	35.1 ± 4.39[a]
Serum osteocalcin *ng/mL*	21.5 ± 4.8	34.5 ± 5.1

Abbreviations are: HD, hemodialysis; CAPD, chronic ambulatory peritoneal dialysis.

[a] Different from low bone turnover, $P < 0.01$, student t test

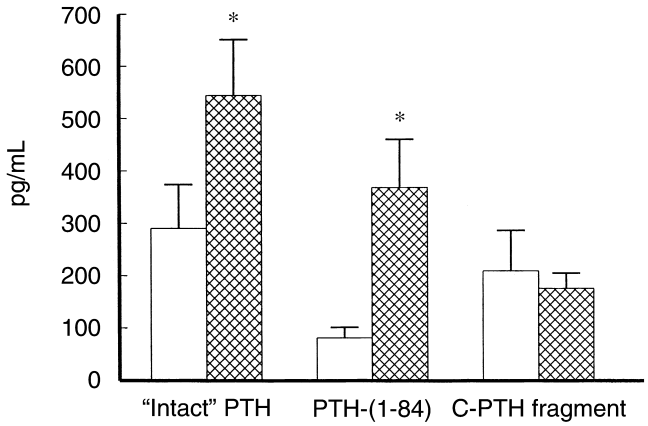

Fig. 1. Mean values of intact parathyroid hormone (PTH), parathyroid hormone (1-84) [PTH-(1-84)], and large carboxyterminal-PTH (C-PTH) fragments (C-PTH fragments) in 51 patients on chronic maintenance dialysis with low bone turnover (□) and high or normal bone turnover (⊠). The asterisk indicates significant differences between high or normal and low bone turnover ($P < 0.01$).

RESULTS

Bone turnover in the studied patients

More than half of the patients exhibited low bone turnover, whereas high or normal bone turnover was found in approximately 45% (Table 1). None of the bone samples exhibited stainable aluminum deposits. Patients with low bone turnover were older than those with high or normal bone turnover (Table 1). Low bone turnover also was found more frequently in diabetics and patients on CAPD; however, this did not reach statistical significance (Table 1). There were no differences in gender distribution, age, number of diabetic patients, duration on dialysis, or biochemical and hormonal parameters between patients on HD and CAPD.

Biochemical and hormonal parameters

Serum calcium and phosphorus levels were similar in patients with various levels of bone turnover (Table 1). Serum BSAP levels were significantly higher in patients with high or normal than low bone turnover (Table 1). Higher serum osteocalcin levels were associated with high or normal bone turnover; however, this did not reach statistical significance (Table 1). Both plasma intact and PTH-(1-84) were significantly higher in patients with high or normal than low bone turnover, whereas the calculated C-PTH fragments were similar (Fig. 1). Patients with low bone turnover had significantly more C-PTH fragments than PTH-(1-84) ($P < 0.001$), whereas patients with high or normal bone turnover had significantly more PTH-(1-84) than C-PTH fragments ($P < 0.001$; Fig. 1), and the PTH-(1-84)/C-PTH fragment ratio was significantly higher in patients with high or normal (range of 0.47 to 14.2) than low bone turnover (range of 0.01 to 0.99; Fig. 2).

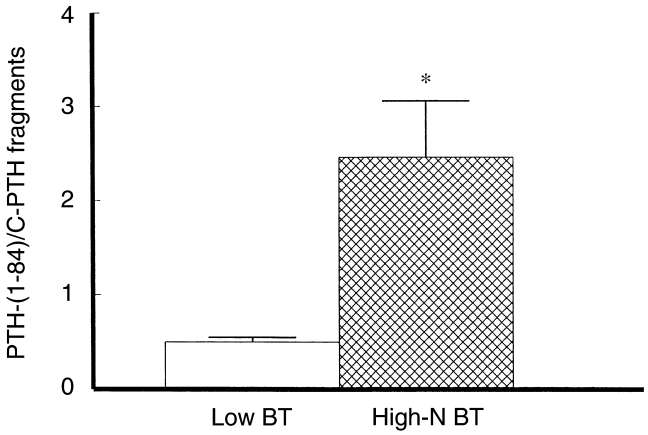

Fig. 2. Mean value of the PTH-(1-84)/C-PTH fragments ratio in 51 patients on chronic maintenance dialysis with low bone turnover (low BT) and high or normal bone turnover (high-N BT). The asterisk indicates significant difference between high or normal and low bone turnover ($P < 0.01$).

Relationship between blood indices and bone turnover

Relationships between plasma intact PTH, PTH-(1-84), PTH-(1-84)/C-PTH fragments, and BSAP and activation frequency (bone turnover) are shown in Figure 3. PTH-(1-84) and PTH-(1-84)/C-PTH fragment ratio correlated best with activation frequency ($r = 0.73$ and 0.68, $P < 0.01$, respectively), whereas intact PTH, osteocalcin, and BSAP had somewhat lower coefficients of correlation (0.51, 0.48, and 0.42, $P < 0.01$, respectively). Serum total and ionized calcium and phosphorus did not correlate with any of the PTH peptides, BSAP, or osteocalcin. All data obtained using BFR/BS as end point yielded results comparable to those obtained with activation frequency.

The PTH-(1-84) levels showed the best relationship

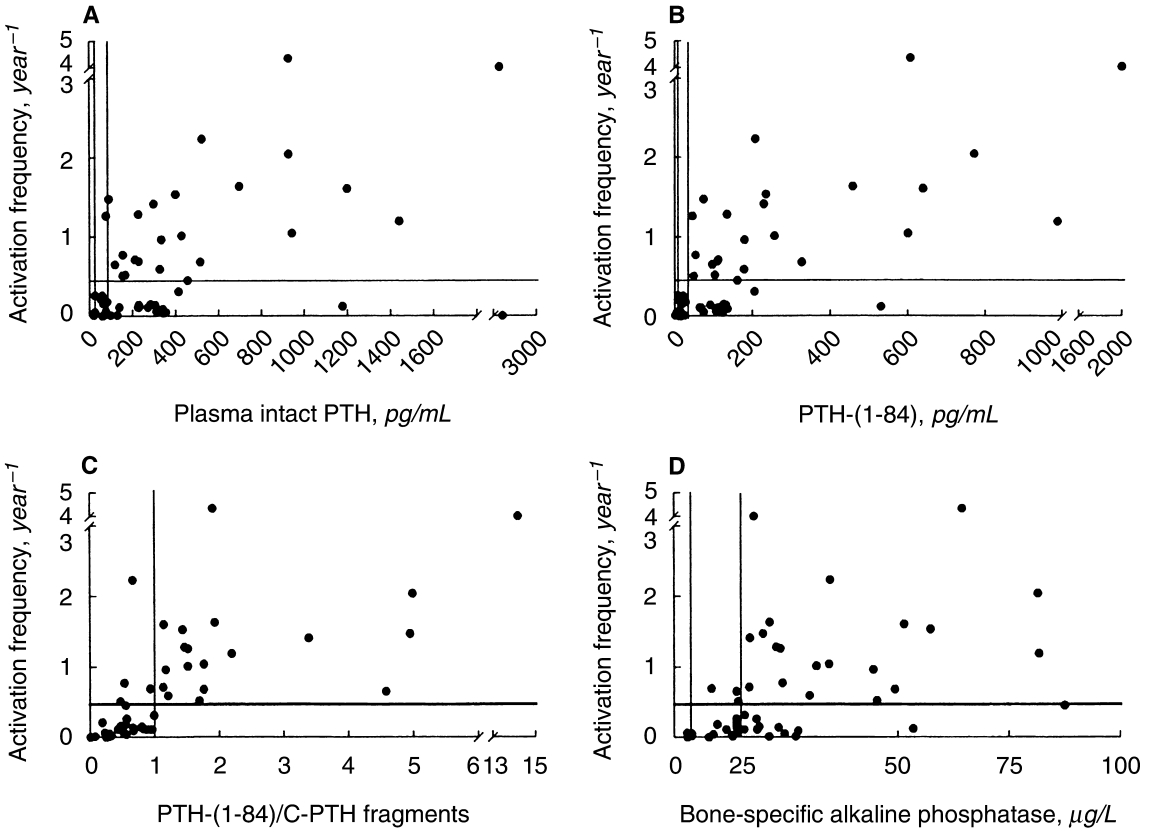

Fig. 3. Relationships between intact PTH, PTH-(1-84), PTH-(1-84)/C-PTH fragment ratio, and bone-specific alkaline phosphatase and bone turnover (activation frequency) in 51 patients on chronic maintenance dialysis.

with bone turnover; however, correlations are driven, at least in part, by the extreme values, whereas the predictive value of a test is determined by the distribution of the intermediate values (slope).

Determination of the best predictor of bone turnover

To determine the factor(s) that predicts bone turnover, we performed logistic regression and post-test probability.

Logistic regression pointed to the PTH-(1-84)/C-PTH fragments ratio as the only parameter predicting bone turnover (activation frequency, Ac.f, $P < 0.001$) with an overall predictability of 88.2%.

$$Ac.f = \frac{1}{1 + e^{-(-5.2+5.5 \text{ ratio})}}$$

Post-test probability parameters for the PTH-(1-84)/C-PTH fragment ratio to predict low bone turnover are shown in Table 2. Their composite results, that is, the ROC curves for PTH-(1-84)/C-PTH fragments ratio as well as for PTH-(1-84), intact PTH, BSAP, and osteocalcin are shown in Figure 4. The area under the curve for the PTH-(1-84)/C-PTH fragment ratio was significantly greater than those of PTH-(1-84) ($P < 0.05$), BSAP ($P < 0.05$), intact PTH ($P < 0.01$), and osteocalcin ($P < 0.01$; Fig. 5). The area under the curve was also significantly greater for PTH-(1-84) than for intact PTH and osteocalcin ($P < 0.05$; Fig. 5). There was no difference between BSAP, osteocalcin, and intact PTH (Fig. 5).

Determination of the best cut-off point

To determine the best cut-off level of the PTH-(1-84)/C-PTH fragment ratio, the Youden indices for the PTH-(1-84)/C-PTH fragment ratio were calculated for every level of the ratio. Given that a perfect cut-off would have a Youden index of 1, the highest Youden index (0.826) was found for a value of PTH-(1-84)/C-PTH fragment ratio of 1 (Fig. 6).

To test how this cut-off point discriminates between levels of bone turnover, we determined the number of patients correctly diagnosed with a PTH-(1-84)/C-PTH fragment ratio above or below 1 (Fig. 3). All 19 patients with a PTH-(1-84)/C-PTH fragment ratio above 1 had normal or high bone turnover. The 32 patients with a ratio less than 1 exhibited low bone turnover except in 4 patients who had either normal ($N = 2$) or high bone turnover ($N = 2$). No other parameters, alone or in

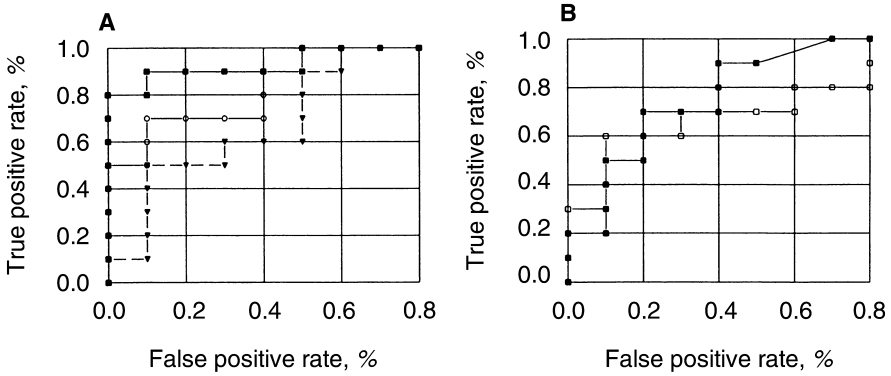

Fig. 4. Receiver-operator characteristics (ROC) curves for the prediction of bone turnover in chronically dialyzed patients. (*A*) Symbols are: plasma PTH-(1-84)/C-PTH fragment ratio (■), plasma PTH-(1-84) (○), and plasma intact PTH (▼). (*B*) Symbols are: serum bone-specific alkaline phosphatase (■) and serum osteocalcin (□).

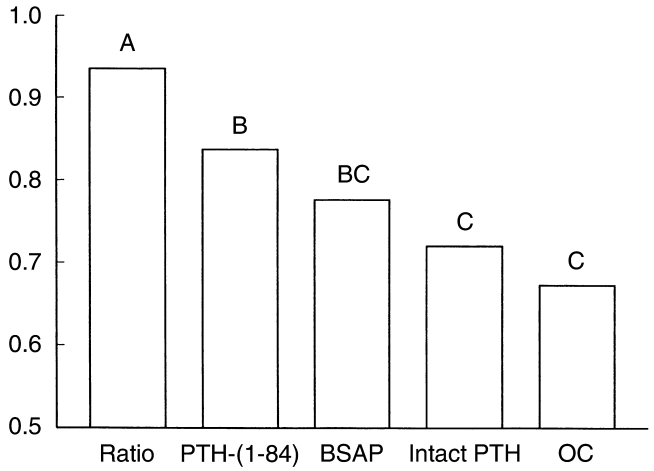

Fig. 5. Area under the curve (AUC) of the receiver-operator characteristics (ROC) curves for PTH-(1-84)/C-PTH fragments ratio, PTH-(1-84), bone-specific alkaline phosphatase (BSAP), intact PTH, and osteocalcin (OC). Values with the same letter are not significantly different.

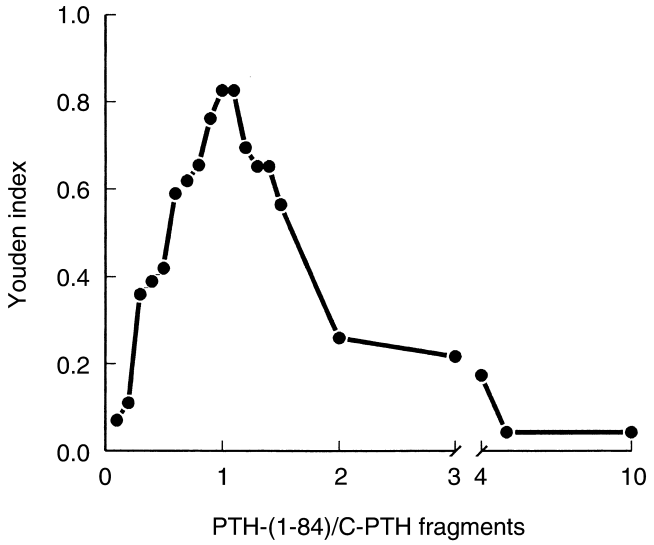

Fig. 6. Youden indices for determination of the optimal cut-off level of the PTH-(1-84)/C-PTH fragments ratio for prediction of bone turnover.

Table 2. Post-test probability of PTH-(1-84)/C-PTH fragments PTH ratio for prediction of low bone turnover

PTH-(1-84)/C-PTH fragments	Sens	Spec	PVP	PVN
	%			
<0.3	35.7	100	100	56.1
<0.4	39.2	100	100	57.5
<0.5	46.4	95.6	92.8	59.5
<0.6	67.8	91.3	90.5	70.0
<0.7	75.0	86.9	87.7	74.1
<0.8	78.6	86.9	88.0	76.9
<0.9	89.3	86.9	89.3	86.9
<1.0	100	82.6	87.5	100
<1.1	100	82.6	87.5	100
<1.2	100	69.5	80.0	100

Abbreviations are: Sens, sensitivity; Spec, specificity; PVP, predictive value for positive results; PVN, predictive value for negative results.

combination, including serum calcium, identified these four patients. When patients with blood intact PTH levels between 100 and 500 pg/mL were analyzed separately, a PTH-(1-84)/C-PTH fragment ratio >1 was also 100% diagnostic for high or normal bone turnover, whereas a ratio <1 was 82% diagnostic for low bone turnover.

Effects of hypercalcemia on the PTH-(1-84)/C-PTH fragments ratio

Induction of hypercalcemia by infusion of calcium gluconate in six patients resulted in the progressive decrease in intact PTH, PTH-(1-84), as well as the PTH-(1-84)/C-PTH fragment ratio with time (Fig. 7). Moreover, there was an inverse relationship between serum-ionized calcium levels and the PTH-(1-84)/C-PTH fragment ratio in individual patients (Fig. 8).

DISCUSSION

The novel information provided by the present study is the superiority of the PTH-(1-84)/C-PTH fragment ratio in predicting bone turnover compared with all other biochemical parameters, alone or in combination, thus supporting our central hypothesis. Bone turnover is reflected by the balance between the relative amount of circulating PTH-(1-84) and large C-PTH fragments. Predominance of circulating active PTH-(1-84) over the

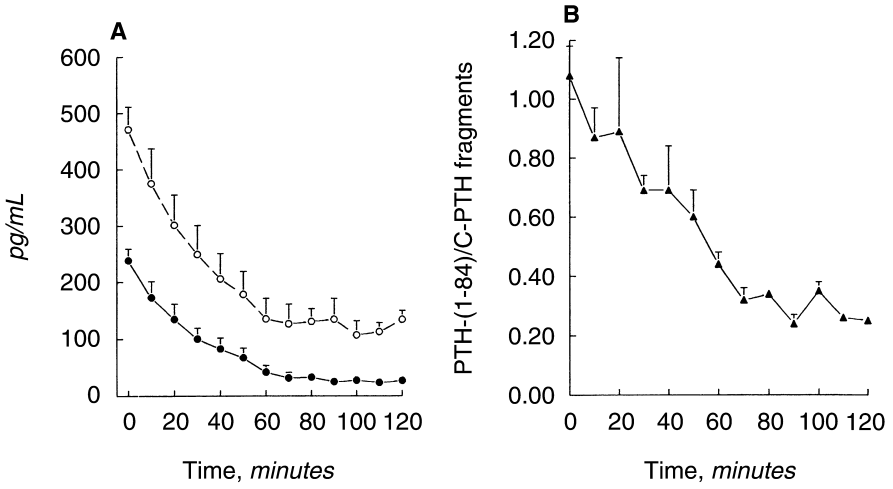

Fig. 7. Changes in PTH peptides during a two-hour calcium gluconate infusion in six patients on dialysis. (*A*) Plasma PTH-(1-84) (●) and intact PTH (○). (*B*) PTH-(1-84)/C-PTH fragment ratio.

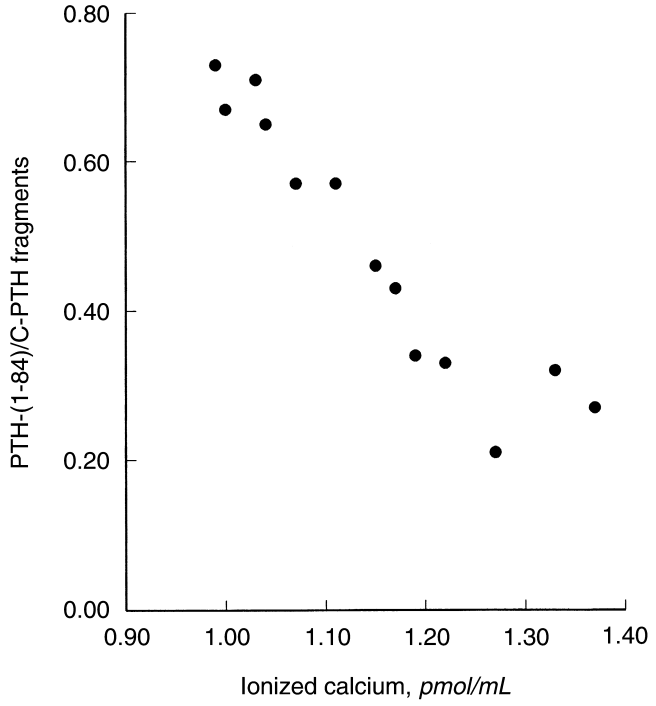

Fig. 8. Relationship between ionized calcium levels and PTH-(1-84)/C-PTH fragments ratio in a patients on dialysis during calcium gluconate infusion.

C-PTH fragments is associated with increased bone turnover, whereas predominance of C-PTH fragments over PTH-(1-84) is most often associated with low bone turnover. The present data lend support to the notion of an antagonistic effect of the C-PTH fragments on PTH-(1-84) action on bone, and extend preclinical observations [24, 32–36] to the dialysis population. The wide range of the obtained results in the PTH-(1-84)/C-PTH fragment ratio (from 0.01 to 14.2) demonstrates that a simple fixed percentage of intact PTH cannot be identified for prediction of the large C-PTH fragments. Even though it is likely that the calculated C-PTH fragments contains several fragments, one can assume that 7-84 PTH is the major antagonist since it was shown to be by far the most potent antagonist among all tested PTH fragments [25]. The remaining scatter between the PTH-(1-84)/C-PTH fragment ratio and bone turnover could be related to different target organ responses at the receptor or post-receptor levels to given levels of PTH.

The mechanism(s) by which the large C-PTH fragments may antagonize the effect of PTH-(1-84) on bone is unclear. It has been speculated that this large amino-terminally truncated fragment (most likely 7-84 PTH) may act as competitive antagonist or regulate the expression or sensitivity of the PTH receptors [11]. Although the 7-34 PTH fragment (which is part of 7-84 PTH) has a binding domain to PTH-1R [48, 49], it is still a poor competitor of 1-34 PTH (which is the active sequence of PTH) for the PTH-1R [32, 33]. This finding renders it unlikely that the antagonistic action of 7-84 PTH on bone effects of PTH-(1-84) is mediated through the PTH-1R. Evidence is accumulating for the expression of functional C-terminal receptors from studies in a variety of skeletally derived cells [13, 18, 25–31]. The basic residues Arg25 and Lys 53 have been recently reported to be critical for effective interaction of PTH-(1-84) with receptors for C-terminal portions of PTH-(1-84) [31]. Further studies are needed to elucidate the precise mode of action of the large C-PTH fragments on bone, in particular if the C-PTH fragments interacts with the PTH-1R, the C-terminal PTH receptor, or another receptor.

Another clinically relevant question concerns the factors that regulate the relative production of PTH-(1-84) and large C-PTH fragments by parathyroid glands. Prevailing serum calcium levels at time of blood sampling did not have an impact on the diagnostic value of the different PTH peptides. This might be due to the cross-sectional design of the study and more certainly to the various degrees of hyperplasia and/or calcium sensitivity of the parathyroid glands in the studied patients ex-

plaining that comparable calcium levels are often observed at different PTH levels [50]. However, our present study demonstrates that changes in serum calcium act as regulators of the relative amount of circulating PTH-(1-84) and C-PTH fragments. These data extend the results of a recent study that demonstrated that plasma levels of PTH-(1-84) decreased more readily than intact PTH with calcium infusion [11]. This is also in agreement with previous in vitro and in vivo studies that showed that bovine parathyroid glands incubated in hypocalcemic medium almost exclusively secrete PTH-(1-84), whereas exposure to hypercalcemic medium results in mostly degraded PTH [51–58]. Taken together, these data suggest that in ESRD patients receiving high doses of calcium on a daily basis, the balance between PTH-(1-84) and C-PTH fragments might favor the synthesis and secretion of the C-PTH fragments, which may result in blunting of PTH-(1-84) actions on bone. It is also of note that replacement of aluminum-containing phosphate binders by calcium salts in the middle 1980s coincided with the emergence of low turnover bone disease without aluminum [59–61]. Further studies are needed to elucidate whether calcitriol or other medications and/or coexistent disease states also have a direct or indirect effect on the PTH-(1-84)/C-PTH fragment ratio.

The present data indicate that interpretation of results of hormone radioimmunoassays may be substantially improved as more bioactive peptides are identified. The level of the measured hormone may reflect not only the entire hormone molecule but also hormone fragments, which may have antagonistic or synergistic effects on the parent molecule. Precedent for this concept is seen with prolactin, which generates a 16 kD N-terminal fragment in mammary tissue or ventral prostate that is then recirculated [62, 63]. This prolactin fragment has its own receptor [64] and has an opposite effect on angiogenesis than prolactin [65]. This, as for PTH, would constitute a useful compensatory mechanism to avoid overactivity of the active hormone.

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